Speciation in the Baboon and its Relation to γ-Chain Heterogeneity and to the Response to Induction of HbF by 5-Azacytidine

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In the baboon (*Papio* species), the two nonallelic γ-genes produce γ-chains that differ at a minimum at residue 75, where isoleucine ($^{I}\gamma$-chain) or valine ($^{V}\gamma$) may be present. This situation obtains in baboons that are sometimes designated as *Papio anubis, Papio hamadryas,* and *Papio papio.* However, in *Papio cynocephalus,* although the $^{I}\gamma$-chains are identical with those in the above mentioned types, the $^{V}\gamma$-chains have the substitutions ala → gly at residue 9 and ala → val at residue 23. The $^{V}\gamma$-chains of *P. cynocephalus* are called $^{V}\gamma^{C}$ to distinguish them from the $^{V}\gamma^{A}$-chains of *P. anubis,* etc. A single cynocephalus animal has been found to have only normal $^{I}\gamma$-chains and $^{I}\gamma^{C}$-chains (that is, glycine in residue 9, valine in 23, and isoleucine in 75). When HbF is produced in response to stress with 5-azacytidine, *P. anubis* baboons respond with greater production than do *P. cynocephalus,* and hybrids fall between. Minimal data on *P. hamadryas* and *P. papio* suggest an even lower response than *P. cynocephalus.* As HbF increases under stress, the ratio of $^{I}\gamma$ to $^{V}\gamma$-chains changes from the value in the adult or juvenile baboon toward the ratio in the newborn baboon. However, it does not attain the newborn value. The $^{V}\gamma^{A}$ and $^{V}\gamma^{C}$-genes respond differently to stress. In hybrids, the production of $^{V}\gamma^{A}$-chains exceeds that of $^{V}\gamma^{C}$-chains. A controlling factor in *cis* apparently is present and may be responsible for the species-related extent of total HbF production. It may be concluded that the more primitive the cell in the erythroid maturation series that has been subjected to 5-azacytidine, the more active is the $^{I}\gamma$-gene.

ONLY AS THE SWITCHOVER from the production of fetal hemoglobin (HbF) to adult type hemoglobin (HbA) occurs during the first 6 mo to a year of life, does the newborn infant with sickle cell anemia or homozygous β^{o}-thalassemia begin to show the stigmata of these genetically inherited diseases. The essentially normal status of such infants at birth can be attributed to the lack of anemia and the presence of a high level of HbF. Because of many reports of decreased morbidity in sickle cell anemia patients with elevated HbF, it is generally supposed that prevention of the postnatal switch or its reversal in the adult would lead to an amelioration of the effects of these diseases. As part of the many studies to determine the nature of the switch mechanism and its control,[1,2] the baboon (*Papio cynocephalus*) has been used and, as a system for study, offers numerous advantages.

The organization of the non-α-globin genes of the baboon is virtually identical with that of man.[3] The two baboon γ-genes produce chains that have either isoleucine ($^{I}\gamma$-chain) or valine ($^{V}\gamma$) at position 75.[4,5] On the basis of the postnatal change in the $^{I}\gamma$ to $^{V}\gamma$ ratio, and by analogy to the human situation, we have recently postulated[6] that the baboon $^{I}\gamma$-gene is on the 5′ side of the $^{V}\gamma$-gene and, therefore, is equivalent to the human $^{G}\gamma$-gene. Stress by hypoxia,[7] phenylhydrazine,[7] or 5-azacytidine[8] produces HbF in the baboon to a degree that is genetically determined.[9]

Although, in our initial studies,[6] we observed only $^{I}\gamma$ and $^{V}\gamma$-chains in baboon HbF, continuing investigation has revealed more and more cases with γ-chains other than $^{I}\gamma$ and $^{V}\gamma$. At first these were considered simply to be instances of a γ-chain variant. Finally, however, it became clear that two species of baboon were involved and that the composition of the colony had been changing. Although *Papio cynocephalus* was the species that was used for early experiments,[7,9] it is now obvious that *Papio anubis* was the species for more recent studies[6] and probably, to a degree, also for other investigations.[8] Hill[10] describes in detail the anatomy and taxonomy of the baboon. Although those phenotypic characteristics that distinguish *Papio cynocephalus* (the yellow baboon) from *Papio anubis* (the black-faced baboon) are evident in the mature animal, such phenotypic differences are not apparent in the newborn baboon: only by classifying the parents or by waiting until the baboon matures can the newborn baboon be classified.

This report is concerned with the delineation of the types of γ-chains not only in *P. cynocephalus* and *P. anubis,* but also in *P. hamadryas* and *P. papio,* with the identification of the sequence differences in the types of γ-chains, with the genetic aspects, with the effect of species on the production of HbF under stimulus, and with the varying relationships of $^{V}\gamma$-type and $^{I}\gamma$-type chains under stimulus.

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MATERIALS AND METHODS

The baboons were part of a colony at the Biological Resources Laboratory of the University of Illinois College of Medicine.

A description has been given[6] of the methods for stressing, blood sampling, shipping of samples from Chicago to Pasadena, analysis for hemoglobin components, and isolation of hemoglobins. Animals that were studied from birth were *P. anubis*. Other anubis animals and all *P. cynocephalus*, the *P. hamadryas*, the *P. papio*, and the hybrids were analyzed only after production of HbF by 5-azacytidine.

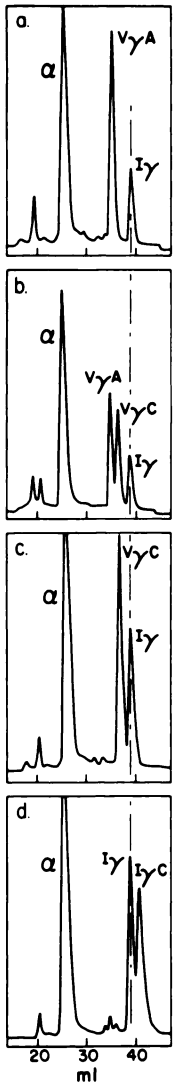
The γ-chain ratio has been determined by either of two methods[11,12] of high performance liquid chromatography (HPLC) that provide equivalent data. HPLC has also been the means for peptide separations[13] and the identification of sequence differences. In instances of modification of procedures, specific information will be provided in the text or figure legend.

RESULTS

Speciation and γ-Chain Heterogeneity

Figure 1 depicts portions of four representative HPL chromatograms that define four types of γ-chain combinations. The heme (not shown) emerges at about 10 ml of effluent volume. These chromatograms were obtained with a new procedure[12] on a Vydac large-pore C_4 column. The separations are far superior to what may be achieved with a Waters μBondapak C_{18} column,[11] although the various types of heterogeneity were apparent on the latter.

The chromatographic pattern in Fig. 1A is representative of all *P. anubis* as well as the single specimens of *P. hamadryas* and *P. papio* that have been studied. The obviously different pattern in Fig. 1C, which is found in most *P. cynocephalus*, indicates that the $^{V}\gamma$-chains are different from those observed in *P. anubis*, *P. hamadryas*, or *P. papio*. Figure 1B demonstrates the pattern in interspecies hybrids of *P. cynocephalus* and either *P. anubis* or *P. hamadryas*. Figure 1D shows the pattern from a single *P. cynocephalus* animal and indicates that it may be homozygous for a variant of the $^{V}\gamma$-gene. In fetus 4505, $^{V}\gamma$-chains of anubis, $^{I}\gamma$-chains, and the variant $^{V}\gamma$-chain of 4778 were present.



anubis: 2057 4446 4510 3010 4449 4525 3200 4450 4528 3851 4461 4549 3950 4467 4612 3987 4470 4615 4005 4471 4625 4047 4476 4626 4048 4484 4430 4485

hybrid: 3760 3955 4611

cynocephalus: 2414 3266 3941 4445 4448 4469 4755 3433

cynocephalus: 4778

Fig. 1. Types of γ-chain heterogeneity and listing of baboons of a specific type. The chromatographic conditions were: Vydac large-pore C_4 column (4.6 × 250 mm) (Cat. no. 214TP54.6, The Separations Group, Box 867, Hesperia, CA 92345); a 30-min gradient from 45% to 55% between 0.1% TFA in 80:20 water-acetonitrile and 40:60 water-acetonitrile and then isocratically at 55%; flow rate 1 ml/min; ambient temperature.

Identification of Sequence Differences in the Various γ-Chains

When HbF from an anubis baboon with the γ-chain heterogeneity in Fig. 1A is examined, the two chains differ only in a valine-isoleucine interchange at position 75. Residue 75 is in tryptic peptide γT-9 (residues 67–76); isolation of the two peptides followed by amino acid analysis gave results as presented in Table 1. These chains, therefore, have been called $^{I}\gamma$ (residue 75 isoleucine) and $^{V}\gamma$ (residue 75 valine).[6]

A portion of the HPL chromatogram of the tryptic digest of globin F from a cynocephalus baboon (Fig. 1C) is depicted in Fig. 2. Peaks with peptides pertinent to this discussion are identified. Peptides $^{I}\gamma$T-9 and $^{V}\gamma$T-9, which define the products of the two types of genes, are present. However, in addition, two kinds of γT-1,2 (residues 1–17, labeled γT-1,2 and γ^{X}T-1,2) and of γT-3 (residues 18–30, labeled γT-3 and γ^{X}T-3) are now apparent. γT-1,2 has the same amino acid composition as the γT-1,2 of $^{V}\gamma$ and $^{I}\gamma$-chains of anubis (Fig. 1A), whereas γ^{X}T-1,2 lacks alanine and has an additional glycine. Consequently, the substitution is in residue 9. Similarly, γ^{X}T-3 has valine instead of the expected alanine at residue 23. The analyses that

Table 1. Amino Acid Analyses Pertinent to the Identification of the Substitutions in the Several Baboon γ-Chains*

Amino Acid	γT-9		γT-1, 2		γT-3	
	$^{I}\gamma$	$^{V}\gamma$	γ	γ^{X}	γ	γ^{X}
Lys	1.02(1)	1.00(1)	1.75(2)	1.96		
His			1.00(1)	1.02		
Arg					0.96(1)	0.98
Asp	1.04(1)	1.01(1)	1.20(1)	1.08	2.02(2)	2.01
Thr	0.93(1)	0.96(1)	2.70(3)	2.81	0.93(1)	0.92
Ser	0.98(1)	1.00(1)	1.20(1)	1.10		
Glu			2.00(2)	2.04	2.05(2)	2.07
Gly	1.02(1)	1.00(1)	1.95(2)	2.95	2.98(3)	2.95
Ala	1.07(1)	1.03(1)	1.10(1)	0	1.07(1)	0
Val	0.94(1)	2.03(2)			2.02(2)	3.05
Ile	1.01(1)	0 (0)	0.95(1)	0.97		
Leu	1.96(2)	1.99(2)	1.00(1)	1.04	0.99(1)	0.99
Phe			0.85(1)	0.99		
Trp			(1)	0.25		

*Figures in parentheses are the theoretical values in residues per peptide.[5]

substantiate these conclusions are given in Table 1 for peptides that were isolated as in Fig. 2 and then purified by further HPLC. The amino acid compositions of other γ-chain peptides were identical in anubis and cynocephalus and matched the data of Nute and Mahoney.[5]

Are the substitutions in the $^{I}\gamma$ and/or the $^{V}\gamma$-chains? In the cynocephalus sample that was used for this study, the HbF had 42% $^{I}\gamma$-chains and 58% $^{V}\gamma$-chains by HPLC of HbF itself. In Fig. 2, the $^{V}\gamma$T-9 peak is greater than the $^{I}\gamma$T-9 peak, and γ^{X}T-3 is greater than the γT-3. Quantitatively by amino acid analysis, there were 10.1 nmole of $^{I}\gamma$T-9 and 13.0 nmole of $^{V}\gamma$T-9 (44% and 56%, respectively). The quantities for γT-1,2 and γ^{X}T-1,2 were 11.6 nmole and 15.9 nmole (42% and 58%), and for γT-3 and γ^{X}T-3 were 11.0 nmole and 18.5 nmole (37% and 63%). We therefore conclude that the two substitutions are in the $^{V}\gamma$-chains of *P. cynocephalus*.

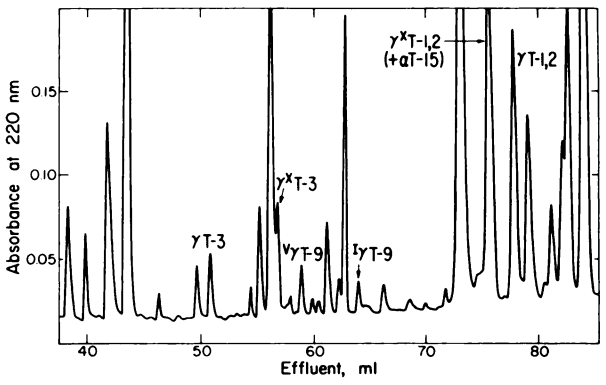


Fig. 2. Separation of tryptic peptides from 0.3 mg of globin F of baboon 4755 by HPLC on an Altex Ultrasphere ODS column (4.6 × 250 mm) with a 0%–45% gradient between 0.1% aqueous TFA and 0.1% TFA in acetonitrile in 120 min at 1 ml/min.

Hence, the $^{I}\gamma$-chains of anubis and cynocephalus are identical, but the $^{V}\gamma$-chains differ at residues 9 and 23 and have been designated $^{V}\gamma^{A}$ and $^{V}\gamma^{C}$ to define the $^{V}\gamma$-chains of anubis and cynocephalus, respectively. In one cynocephalus (baboon 3433), the $^{V}\gamma^{C}$ to $^{I}\gamma$ ratio is the reverse of that of Fig. 1C; further discussion of this animal will be made later.

As anticipated, hybrids (Fig. 1B) have the common $^{I}\gamma$-chain as well as the $^{V}\gamma^{A}$ and $^{V}\gamma^{C}$-chains (data not presented). Data in Fig. 1B derive from baboon 3760, which also had a silent mutation in the β-chain (β13ala → thr; Hb Papio B^{11}).

Only a single cynocephalus baboon (4778) has had the pattern of γ-chains as seen in Fig. 1D. When the tryptic digest of HbF from 4778 was examined by the same procedure as in Fig. 2, the structure became obvious immediately: 4778's digest contained *no* $^{V}\gamma$T-9; that is, only isoleucine is at residue 75. However, both kinds of γT-1,2 and γT-3 were present. Thus, this baboon has two kinds of $^{I}\gamma$-chains; one is the common $^{I}\gamma$-chain and the other, instead of alanine, has glycine and valine at residues 9 and 23, respectively. Therefore, it has been called $^{I}\gamma^{C}$. If this baboon has its full complement of two γ-genes per chromosome, then it is homozygous for the normal 5′ $^{I}\gamma$ and the variant 3′ $^{I}\gamma^{C}$-genes. Data from fetus 4505, which by HPLC had $^{V}\gamma^{A}$, $^{I}\gamma$, and $^{I}\gamma^{C}$-chains, substantiate these conclusions and are summarized in Table 2. The second column gives the percentage of the chains from HPLC of the HbF. The remaining columns then list the percentages of expected peptides from each of the three pairs of distinguishing peptides. The last two lines show the sum of each of the expected peptides and compare these values with the percentages found.

Table 3 summarizes the sequence differences in the four types of baboon γ-chains.

Nute and Mahoney[5] reported the identical sequence that we have found in anubis for a cynocephalus animal. There is, in fact, no conflict. Nute (personal communication) classified according to Buettner-Janusch,[14] who considers both cynocephalus and anubis to be a single species. The Primate Center at Seattle,

Table 2. Correlation of Peptides Expected and Determined in the Tryptic Digest of HbF From Baboon 4505

Chain Type	Percent Chain	Percent Peptides Expected in Digest*					
		$^{V}\gamma$T-9	$^{I}\gamma$T-9	γT-1, 2	γ^{X}T-1, 2	γT-3	γ^{X}T-3
$^{V}\gamma^{A}$	20	20		20		20	
$^{I}\gamma$	62		62	62		62	
$^{I}\gamma^{C}$	18		18		18		18
Sum		20	80	82	18	82	18
Found		21	79	80	20	78	22

*The totals for any pair of peptides equals 100%.

Table 3. Sequence Differences in Four Types of Baboon γ-Chain

Chain	Residue 9	Residue 23	Residue 75	Species
$^{V}\gamma^{A}$	Ala	Ala	Val	*anubis*
$^{V}\gamma^{C}$	Gly	Val	Val	*cynocephalus*
$^{I}\gamma$	Ala	Ala	Ile	*anubis* and *cynocephalus*
$^{I}\gamma^{C}$	Gly	Val	Ile	*cynocephalus*

from which Nute and Mahoney's animal was obtained, listed the baboon as *P. cynocephalus anubis.*

HbF Production by Injection of 5-Azacytidine

The response of the individual baboon to stress has been shown to involve genetic factors.[9,15] The magnitude of the response to phenylhydrazine stress divides the various baboons into low, intermediate, and high responders. The maximum percentage of HbF that is produced by baboons of various types after injection of 5-azacytidine is plotted in Fig. 3.

In these experiments, the baboons were bled 5 days a week for 1 or 2 wk to reduce the hematocrit to approximately 0.20 liter/liter. Intravenous injection of 5-azacytidine at 4 mg/kg was done for 5 days each week, as bleeding was continued to maintain the reduced hematocrit. Despite the fact that the total number of injections in individual cases ranged from 10 to 21, this variation is not responsible for the significant range of results within a species. The open circles denote the data from 10 injections, and these data scatter both high and low within the range of a given species.

Although within species there is a considerable range of response to 5-azacytidine (Fig. 3), and although one may not in all cases be able to classify an individual baboon on the basis of response, yet the response appears to be strongly species specific. On the average, the anubis baboons produce more HbF than do the cynocephalus, and the hybrids fall between. The few data from *P. hamadryas* and *P. papio* suggest an even lower response than that of *P. cynocephalus.* Some substantiation of the low response of *P. hamadryas* comes from the fact that the hybrid of lowest response (3760) is a cynocephalus-hamadryas cross,

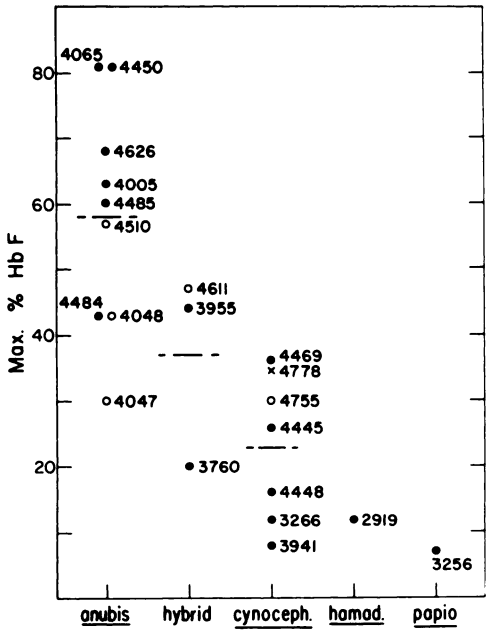


Fig. 3. Maximum percentage of HbF in various species of baboons after stress with 5-azacytidine. The dashed lines are the averages.

whereas the other two hybrids are anubis-cynocephalus.

The value denoted by "X" in Fig. 3 is that for 4778, the baboon with $^{I}\gamma$ and $^{I}\gamma^{C}$-chains.

Although most of the animals whose response to phenylhydrazine was the basis of classification into high, intermediate, and low responders[15] are no longer available for study, the few for whom response to both phenylhydrazine and 5-azacytidine may be compared do show good correlation between the two procedures. We conclude that response to such stresses is species specific.

The various data are summarized in Table 4.

Variation in the Ratio of γ-Chains With Stress

In the baboon, as in the human, the ratio of γ-chains at birth changes over the first few postnatal months.[6] The data from anubis baboons showed an $^{I}\gamma$ to $^{V}\gamma^{A}$ ratio of about 3:2 at birth, with a change to approxi-

Table 4. Summary of Data From Various Species of Baboons

Species	Chains	Chain Pattern	Response to 5-Azacytidine	Percent HbF	
				Average Increase	Range
anubis	$^{V}\gamma^{A} + {}^{I}\gamma$	Fig. 1A	High	58	30–81
hamadryas	$^{V}\gamma^{A} + {}^{I}\gamma$	Fig. 1A	Very low	12*	
papio	$^{V}\gamma^{A} + {}^{I}\gamma$	Fig. 1A	Very low	7*	
cynocephalus	$^{V}\gamma^{C} + {}^{I}\gamma$	Fig. 1C	Low	24	7–36
	$^{I}\gamma^{C} + {}^{I}\gamma$	Fig. 1D	Low	35*	
anu.-cyno. hybrid	$^{V}\gamma^{A} + {}^{V}\gamma^{C} + {}^{I}\gamma$	Fig. 1B	Intermediate	37	20–47

*One value only.

mately 1:2 in the HbF at about 6 mo of age. From this information, it was concluded that the $^{I}\gamma$-gene is probably equivalent to the human $^{G}\gamma$-gene and 5′ to the $^{V}\gamma^{A}$-gene.

After the postnatal change had occurred, stress with 5-azacytidine altered the ratio toward the newborn ratio; two examples with anubis baboons have been described.[6] At present, the response of 30 animals has been evaluated; in some, more than one test has been made. Not only intravenous but also subcutaneous injection as well as oral administration has been used, and other compounds have been given simultaneously.

Further representative examples of the change in the γ-chain ratio as the HbF increases under stress are presented in Fig. 4, which shows results not only from anubis, but also from cynocephalus and a hybrid between these species. As may be seen, in each case, the percent $^{I}\gamma$-chain at the start of the experiment approximates about 30%, which is an average for the HbF in the adult or juvenile animal. As HbF increases, the percent $^{I}\gamma$-chain also rises, although in no case has it reached about 60% as in the newborn.

Although these data are representative, and although about 30% is the average $^{I}\gamma$-chain amount in the animal at the start of an experiment, a considerable range may be seen in individual animals. The lowest $^{I}\gamma$-chain amount that has been observed initially is 7% and the highest is 42%. In the former, the $^{I}\gamma$-gene or genes have virtually shut down. On the average, as the HbF increases, the amount of $^{I}\gamma$-chains will rise about 15%. However, again there is an individualistic response: the greatest increase has been 26% (from 7% to 33%), but in 3 animals the percent $^{I}\gamma$-chain remained the same within experimental error. The final percentage of $^{I}\gamma$-chain approaches 50 only when the initial percentage approximates 30.

Differential Behavior of the $^{V}\gamma^{A}$ and $^{V}\gamma^{C}$-Genes to 5-Azacytidine

Although the chain ratio in cynocephalus changes under stress (Fig. 4), quantitative data are fewer than for anubis because the earlier procedure for chain separation[11] was rather ineffective in separating the $^{I}\gamma$ and $^{V}\gamma^{C}$-chains. We have already noted the lessened response of cynocephalus baboons to stress by 5-azacytidine (Fig. 3). This difference in HbF response is also reflected as a differential response of the $^{V}\gamma^{A}$ and $^{V}\gamma^{C}$-genes in the hybrids. Figure 5 presents not only the change in the $^{I}\gamma$-chains of anubis-cynocephalus hybrid 4611, but also shows the change in $^{V}\gamma^{A}$ and $^{V}\gamma^{C}$-chains. Of the total $^{V}\gamma$-chains, the $^{V}\gamma^{A}$-chains are 54%–58% in the various samples. The differential response of $^{V}\gamma^{A}$-genes in anubis and of $^{V}\gamma^{C}$-chains in cynocephalus probably is unrelated to the sequence differences, because hamadryas and papio, which are low responders, have the $^{V}\gamma^{A}$-genes of the high responding anubis.

Genetic Relationships

The γ-chain composition distinguishes anubis, hamadryas, and papio from cynocephalus and detects hybrids. In retrospect, however, it is not always possible to substantiate this type of identification by examination of the baboon, because some animals that were studied earlier are no longer in the colony. Despite the identifying characteristics that Hill gives,[10] gradations may make it difficult to make a definite identification or a hybrid may have so definitely the characteristics of one species that it would be misidentified in the

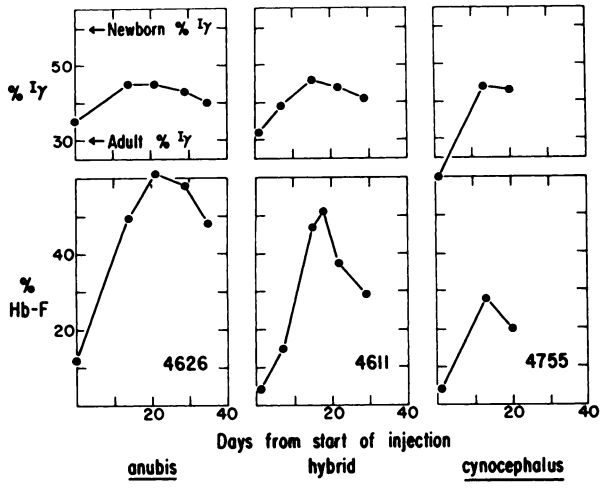


Fig. 4. Increase in HbF and related change in the percent $^{I}\gamma$-chain in representative anubis, cynocephalus, and hybrid baboons.

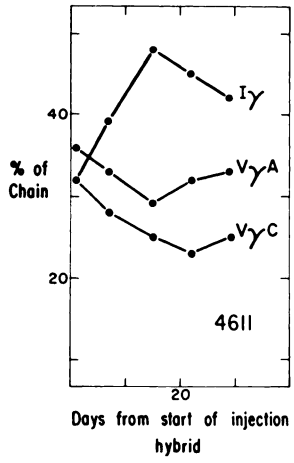


Fig. 5. Time course of the change in γ-chain composition in anubis-cynocephalus hybrid 4611 in response to 5-azacytidine stress.

absence of the data about the γ-chains. Some animals have been born in the colony and others are imports. For example, 3955 is a hybrid, although phenotypically both parents are cynocephalus; presumably one is a hybrid. Baboon 3433 produces only $^{I}\gamma$ and $^{V}\gamma^{C}$-chains of a cynocephalus, yet the father phenotypically is an anubis and the mother is unclassified.

Three baboons are of especial interest: 4778 produces only $^{I}\gamma$ and $^{I}\gamma^{C}$-chains, fetus 4505 had $^{V}\gamma^{A}$, $^{I}\gamma$, and $^{I}\gamma^{C}$-chains, and 3433 has $^{V}\gamma^{C}$ and $^{I}\gamma$-chains in the reverse ratio of other cynocephalus baboons. It may be assumed that the anubis baboons normally would be homozygous for the nonallelic γ-genes; thus, $^{I}\gamma$, $^{V}\gamma^{A}$/ $^{I}\gamma$, $^{V}\gamma^{A}$. Similarly, the cynocephalus baboons would have $^{I}\gamma$, $^{V}\gamma^{C}/^{I}\gamma$, $^{V}\gamma^{C}$.

The mother (4439) and the father (4380) of 4778 are phenotypically cynocephalus. If 4778 is homozygous at both loci ($^{I}\gamma$, $^{I}\gamma^{C}/^{I}\gamma$, $^{I}\gamma^{C}$), both parents, at a minimum, must be heterozygotes of the type $^{I}\gamma$, $^{I}\gamma^{C}/^{I}\gamma$, $^{V}\gamma^{C}$, although the expression in 4778 could result if one parent were—, $—/^{I}\gamma$, $^{V}\gamma^{C}$, in which the γ-genes on one chromosome were deleted or inactive (other parental arrangements with deleted or inactive genes, of course, can be designed). However, 4778's response to stress is normal for a cynocephalus baboon, and the full complement of γ-chains may well be present. Baboon 4380 is also the father of fetus 4505, whereas the mother (4505) is an anubis. In all probability, fetus 4505 had the genic arrangement $^{I}\gamma$, $^{V}\gamma^{A}/^{I}\gamma$, $^{I}\gamma^{C}$.

The fact that 4778 apparently is homozygous and fetus 4505 was heterozygous for the $^{I}\gamma^{C}$-gene indicates some frequency of the gene in the cynocephalus population (despite the fact that 4380 is the father of both). The quantity of product and the amino acid sequence suggest that the $^{I}\gamma^{C}$-gene is in the position of the usual $^{V}\gamma$-gene and is under the same control as a $^{V}\gamma$-gene. The human $^{A}\gamma^{T}$-gene is present in considerable frequency,[16,17] and it, too, involves position 75 in which threonine replaces isoleucine. Perhaps the baboon $^{I}\gamma^{C}$-gene is analogous to the human $^{A}\gamma^{T}$-gene. One can but speculate on the origin of the $^{I}\gamma^{C}$-gene. By a single point mutation, isoleucine would substitute for valine. Nonhomologous crossover, however, would not produce the necessary genic arrangement. On the other hand, so-called gene "conversion," as postulated by Slightom et al.[18] could yield $^{I}\gamma$, $^{I}\gamma^{C}$. Such a gene conversion could also form $^{V}\gamma^{A}$, $^{V}\gamma^{C}$, which if inherited with $^{I}\gamma$, $^{V}\gamma^{C}$ of the common cynocephalus would, in fact, mimic an anubis-cynocephalus hybrid. However, the ratio of the products of a $^{V}\gamma^{A}$, $^{V}\gamma^{C}/^{I}\gamma$, $^{V}\gamma^{C}$ combination should differ from those of a $^{I}\gamma$, $^{V}\gamma^{A}/^{I}\gamma$, $^{V}\gamma^{C}$ union.

Various genic arrangements may be postulated to explain the reverse of the common cynocephalus ratio of γ-chains in baboon 3433; possibilities are $^{I}\gamma$,—/$^{I}\gamma$, $^{V}\gamma^{C}$ or $^{I}\gamma$, $^{I}\gamma/^{I}\gamma$, $^{V}\gamma^{C}$. Nonhomologous crossing-over or gene conversion could be responsible.

DISCUSSION

There is controversy over the proper classification of baboons. Hill[10] distinguishes five living species and subdivides some species into several races. Buettner-Janusch[14] considers all baboons, with the possible exception of *Papio hamadryas* (the sacred Egyptian baboon) to be a single species to be termed *Papio cynocephalus*. Nevertheless, regardless of what the classification may be, it is now apparent that significant differences exist in the γ-genes of several species (subspecies, races?) and in the response of these genes to abnormal stress.

The $^{I}\gamma$-gene is common to all baboons that we have examined, and, from all available data, it is the 5′ baboon γ-gene and analogous to the human $^{G}\gamma$-gene. If, in any case, some modification of this gene is present in one or more of the baboons examined, its product mimics either the normal product or that of one of the other γ-genes.

In anubis, the sole difference in product between the $^{I}\gamma$-gene and the $^{V}\gamma^{A}$-gene, its 3′ nonallelic partner, is the substitution of valine for isoleucine in position 75. The greater difference of three residues between the $^{I}\gamma$ and $^{V}\gamma^{C}$-chains of cynocephalus has shown itself further in the other modifications, one of which is the $^{I}\gamma^{C}$-chain.

It is unlikely that the difference in sequences of the $^{V}\gamma$-chains in anubis and cynocephalus is responsible for the varied effect of stress with 5-azacytidine. Thus, hamadryas and papio, with apparently the same γ-genes as anubis, produce little HbF with 5-azacytidine. Previous study[6] of the postnatal decline of HbF and the change in γ-chain ratio has been limited to anubis newborn. It remains to be seen whether the postnatal changes in a cynocephalus newborn will differ. If so, they may provide insight into the control of the switch from HbF to HbA. Even in the same species, 5-azacytidine does not produce the same effect (Fig. 3). Perhaps the low responders of anubis and the high responders of cynocephalus are heterozygous for a controlling locus that is homologous to the F-cell response locus, as postulated by Dover et al.[19]

The difference between anubis and cynocephalus baboons is also reflected in a differential response of the $^{V}\gamma^{A}$ and $^{V}\gamma^{C}$-genes in interspecies hybrids. Because more $^{V}\gamma^{A}$ than $^{V}\gamma^{C}$-chains are produced in hybrids, the species differences in the expression of γ-genes must be related to a locus in *cis* (controlling sequences). Previous data have indicated that the differential HbF response is due to differences in the number of cells

that contain HbF (F cells);[9] high responders have twice as many F cells as low responders.

At what stage in erythrocyte differentiation is 5-azacytidine effective? In attempting to answer this question, it is assumed that the kinetics of erythroid maturation in the baboon is comparable to that in the human. Approximately 4–6 days are required for cell division and differentiation from the pronormoblast to the erythrocyte.[20] If 5-azacytidine acts by producing hypomethylation in the DNA, this must occur in the first half of this process because DNA synthesis take place only during the first half of the time period. In our experiments, after initial bleeding to lower the hematocrit, the next sample after the beginning of 5-azacytidine treatment usually is taken a week later. Although there are exceptions, an increase in the percent $^{1}\gamma$-chains in the HbF is evident in 90% of the experiments. If the initial HbF is minimal, and if the increase in HbF is severalfold at least, then the new HbF is not significantly diluted by the initial HbF, and percent $^{1}\gamma$-chain measures the average over the period. Because of the continuing rise in percent $^{1}\gamma$-chain with HbF, there must be increasing reactivation of the $^{1}\gamma$-gene. Although measurement of the percent $^{1}\gamma$-chain in the HbF of the peripheral blood determines the accumulation of $^{1}\gamma$-chains over a time period, an instantaneous measure can be made by incubation with a radioactive amino acid. When this was done with an anubis baboon, the percent $^{1}\gamma$-chain by radioactivity at maximum HbF was 45%, whereas it was 35% in the peripheral blood. Further experiments of this type are in progress. The radioactive incorporation measures the synthesis in the reticulocyte. Prior to 5 days after the beginning of injection of 5-azacytidine, the reticulocytes will have derived from cells at different stages in the maturation series. In the experiment already mentioned, there is increasing divergence with time between percent $^{1}\gamma$ as measured by radioactivity and in the peripheral blood. Consequently, it may be concluded that the earlier the cell type that has been subjected to 5-azacytidine, the more active is the $^{1}\gamma$-gene. The more active the $^{1}\gamma$-gene is, the more has the control of production of γ-chains reverted to the prenatal and newborn state, where 60% $^{1}\gamma$-chains are present in the peripheral blood. In none of the experiments has the percent $^{1}\gamma$ reached that of the prenatal and newborn state. However, it would appear that the influence of 5-azacytidine is continued back beyond the pronormoblast: not only is treatment continued beyond the 5-day maturation period, but HbF and percent $^{1}\gamma$-chains also continue to increase.

The long-held opinion that prevention of the postnatal switch from HbF to HbA or its reversal would benefit the individual with sickle cell anemia or β^{o}-thalassemia has prompted much effort to learn how the switch is controlled. 5-Azacytidine is effective in producing HbF in the baboon, although the degree is dependent on genetic factors, as has been shown previously[9,15] and has been further delineated here. Despite the potential carcinogenicity of 5-azacytidine, preliminary human trials with 5-azacytidine have been made.[21-24] The obvious increase in HbF in these cases was modest and did not approach the large production in some baboons. It may be, of course, that genetic factors of the type that influences the response in baboons also occur in humans, or perhaps the dosage was inadequate. It is not necessary to enumerate here the many problems that must be solved before 5-azacytidine or some other compound could be used in the long term to ameliorate effectively the adverse expression of sickle cell anemia or β^{o}-thalassemia. However, the baboon continues to provide an excellent animal model for further study.

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